PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference RPS/60637001	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/GB 03/04768	International filing date *(day/month/year)* 03.11.2003	Priority date *(day/month/year)* 01.11.2002
International Patent Classification (IPC) or both national classification and IPC C12N15/10		
Applicant NORCHIP AS		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 7 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
01.06.2004	11.03.2005
Name and mailing address of the international preliminary examining authority: European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Authorized Officer Tiede, R Telephone No. +31 70 340-1090

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB 03/04768

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-28 as originally filed

Claims, Numbers

37, 38 as originally filed

1-36 received on 03.02.2005 with letter of 01.02.2005

Drawings, Sheets

1/3-3/3 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

☐ the language of publication of the international application (under Rule 48.3(b)).

☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

☐ contained in the international application in written form.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

☐ the description, pages:

☒ the claims, Nos.: 37-39

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-36
	No:	Claims	
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-36
Industrial applicability (IA)	Yes:	Claims	1-36
	No:	Claims	

2. Citations and explanations

   see separate sheet

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-36
	No:	Claims	
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-36
Industrial applicability (IA)	Yes:	Claims	1-36
	No:	Claims	

2. Citations and explanations

   see separate sheet

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following documents:

D1: US 2002/068357 A1 (MATHIES RICHARD A ET AL) 6 June 2002 (2002-06-06)
D2: US 2001/014850 A1 (GILMANSHIN RUDOLF ET AL) 16 August 2001 (2001-08-16)
D3: US-A-5 304 487 (WILDING PETER ET AL) 19 April 1994 (1994-04-19)
D4: US-A-4 761 381 (BLATT JOEL M ET AL) 2 August 1988 (1988-08-02)

1 Claim 1 does not meet the requirements of Article 6 PCT in that the matter for which protection is sought is not clearly defined. The functional statement: "dimensioned to impede the flow ... so as to effect shearing of nucleic acid molecules" does not enable the skilled person to determine which technical features are necessary to perform the stated functions. This feature has only a meaning for and in relation to a specific use and defines no device feature as such. This functional feature depends also on the nature of the transport fluids, their flow rates and the specific nucleic acid molecules to be fragmented. This feature therefore relates to the use of such a device rather than to the device features which enable such use. The intended scope of claim 1 with respect to the dimensions of the outlet port is therefore unclear.

2 D1, as closest prior art, discloses the physical fragmentation of Nucleic Acid molecules by "forcing it through restricted size flow passages, e.g. apertures having a cross sectional dimension in the micron or submicron range" (D1, paragraph 136). Additionally, D1 discloses the use of obstacles for fragmentation. D1 cites as an example of the general layout of the device as being that of D3 (which is included by reference). Consequently, subject-matter of claim 1 differs from D1 in that the side walls taper towards the inlet port.

2.1 This additional technical feature solves the problem of air bubbles which might be trapped during filling the chamber (page 7 of the description), i.e. to avoid such air bubbles.

2.2 The problem is well known in the art of microfluidic devices, see for example introduction of D4 (column 1, line 55 to column 2, line 7). D4 solves the same problem with the same solution, namely by avoiding sharp corners and by tapered side walls (e.g. D4, fig. 6, column 7, lines 37-43).

2.3 The combination of a fragmentation cell with features to avoid bubble formation in the cell leads to no surprising effects, which would go beyond that which has already been known from D4 and/or D1. The problem solved in view of D1 (point 2.1) is omnipresent in microfluidic devices and the problem seems to be unrelated to the problem of fragmenting nucleic acid molecules. The person skilled in the art would therefore combine the teachings of D1 and D4 and arrive at a device as claimed in claim 1 without exercising inventive steps.

2.4 Subject-matter of claim 1 is therefore not inventive (Article 33(3) PCT).

3 Dependent claims 2-24 do not contain any features which, in combination with the features of any claim to which they refer, meet the requirements of the PCT in respect of novelty and/or inventive step, see document D1 in particular passage 136 and other passages cited in the search report.

4 D1 discloses also the use of said device as claimed in claim 31 and 32; a kit as claimed in claim 33 and 34; and a process of fragmenting nucleic acids in a fluid sample as claimed in claims 35 and 36 (see citations as mentioned above). Subject-matter of claims 31-36 is therefore equally not inventive (Article 33(3) PCT).

5 Furthermore, D2 discloses (D2: passages 114-147; fig. 8 and 9): A variety of different forms of channel constrictions and obstacles in microfluidic devices, D2 also discloses to repeat said constrictions and obstacles in a serial fashion (paragraph 119). The teaching of D2 is directed to avoid fragmentation in said structures. However, inevitably fragmentation does occur at least in part (paragraphs 114). Lacking any further technical differences with respect to the construction of the

device which would influence the fragmentation process, and as fragmentation will occur, the cell of D2 can therefore be considered as being a fragmentation cell. In other words, with regard to the fragmentation of molecules D2 differs from claim 1 only in operational (use related) rather than constructional features. Apart from the differences as to how to use said device in D2, present claim 1 differs from D2 in that no tapered side walls at the inlet port are disclosed. The same reasons as outlined under point 2.2 and 2.3 can be levelled against claim 1 with respect to the combination of D2 and D4.

5.1 Consequently, subject-matter of claims 1-30 are not inventive with respect to Article 33(3) PCT.

6 Similarly, D3 discloses similar technical features as D1 and D2 with respect to claims 1-5,7,10,15-27 and 33-39 (see passages as cited in the International Search Report). The subject-matter of these claims is thus, when again combined with the teachings of D4 (see point 2.1 and 2.2), not inventive with respect to Article 33(2) PCT.

CLAIMS:

1. A microfabricated device for fragmenting nucleic acids present in a fluid sample, the device comprising an inlet port, a fragmentation cell, and an outlet port downstream from said inlet port, said cell being in fluid communication with said ports, and wherein said outlet port is dimensioned to impede the flow of a fluid sample out of said cell so as to effect shearing of nucleic acids molecules therein.

2. A microfabricated device as claimed in claim 1, wherein the width of the fragmentation cell abruptly decreases at the outlet port.

3. A microfabricated device as claimed in claim 1 or claim 2, wherein the outlet port comprises a constriction, preferably having a width in the range of from 1 to 100 μm, more preferably from 5 to 50 μm.

4. A microfabricated device as claimed in any one of the preceding claims, wherein the fragmentation cell comprises a chamber having a bottom wall in which is formed the outlet port, the bottom wall being generally perpendicular to the direction of flow of fluid through the outlet port.

5. A microfabricated device as claimed in claim 4, wherein the outlet port is formed in approximately the middle of the bottom wall.

6. A microfabricated device as claimed in any one of the preceding claims, wherein the fragmentation cell has the shape of an irregular polygon (preferably an irregular) hexagon) with an essentially straight bottom wall in which the outlet port is formed at approximately the mid point, and wherein the bottom wall is substantially perpendicular to the longitudinal axis of the outlet port.

7. A microfabricated device as claimed in any one of claims 4 to 6, wherein the fragmentation cell has a top wall in which the inlet port is formed, and side walls which extend from the top wall to the bottom wall.

8. A microfabricated device as claimed in claim 7, wherein the side walls taper inwardly to meet the inlet port.

9. A microfabricated device as claimed in claim 7 or claim 8, wherein the side walls taper inwardly to meet the outlet port.

10. A microfabricated device as claimed in any one of claims 4 to 7, wherein the bottom wall is adjacent and substantially perpendicular to two lower side wall portions.

11. A microfabricated device as claimed in claim 10, wherein the upper portions of the side walls taper inwardly to meet the inlet port.

12. A microfabricated device as claimed in any one of the preceding claims, wherein side walls or portions thereof

next to or adjacent the inlet port subtend an angle of less than 90 degrees to the longitudinal axis of the inlet port.

13. A microfabricated device as claimed in any one of claims 1 to 5, wherein the fragmentation cell comprises a bottom wall in which the outlet port is formed at approximately the mid point, the bottom wall being substantially perpendicular to the longitudinal axis of the outlet, and side walls which converge or taper inwardly to meet the inlet port.

14. A microfabricated device as claimed in any one of claims 1 to 5 and 13, wherein the fragmentation cell is generally pear shaped with an essentially straight bottom wall in which the outlet port is formed at approximately the mid point, the bottom wall being substantially perpendicular to the longitudinal axis of the outlet, and wherein the bottom wall is connected by curved walls to side walls, which converge or taper inwardly to meet the inlet port.

15. A microfabricated device as claimed in any one of the preceding claims, wherein the device further comprises an obstacle located in the cell in the direct path between the inlet and outlet ports.

16. A microfabricated device as claimed in claim 15, wherein the space between sides of the obstacle and sides of the cell defines a bifurcated path for the fluid sample.

17. A microfabricated device as claimed in claim 15 or claim 16, wherein the obstacle is shaped so that the flow path of a fluid sample in a region adjacent the outlet port

is substantially perpendicular to the longitudinal axis of the outlet.

18. A microfabricated device as claimed in any one of claims 15 to 17, wherein the obstacle is in the form of a generally triangular obstacle, with its three sides substantially parallel to the bottom wall and side walls of the cell, the space between the sides of the obstacle and the sides of the cell defining a bifurcated path for the fluid sample.

19. A microfabricated device as claimed in any one of the preceding claims, wherein the fragmentation cell is asymmetric about the horizontal axis and substantially symmetric about the longitudinal axis, the longitudinal axis being essentially coincident with the direction of flow.

20. A microfabricated device as claimed in any one of the preceding claims, further comprising an access channel in fluid communication with the inlet port.

21. A microfabricated device as claimed in any one of the preceding claims, further comprising collection means in fluid communication with the outlet port.

22. A microfabricated device as claimed in any one of the preceding claims, further comprising means for effecting flow of a sample into the inlet port, through the fragmentation cell and out of the outlet port.

23. A microfabricated device as claimed in claim 22, wherein said means for effecting flow comprises one or more pumps.

24. A microfabricated device as claimed in claim 22, wherein said means for effecting flow comprises one or more variable volume chambers in communication with the inlet port and/or outlet port, wherein altering the volume of the variable volume chamber(s) effects and/or restricts flow of a fluid sample into and/or out of the fragmentation cell.

25. A microfabricated device as claimed in any one of the preceding claims which comprises a substrate and an overlying cover, the fragmentation cell being defined by a recess in a surface of the substrate and the adjacent surface of the cover.

26. A microfabricated device as claimed in claim 25, wherein the substrate is formed from silicon and the overlying cover from glass.

27. A microfabricated device as claimed in claim 26, wherein the glass cover is anodically bonded to the silicon substrate, optionally through an intermediate silicon oxide layer formed on the surface of the substrata.

28. A microfabricated device as claimed in any one of the preceding claims which comprises at least first and second fragmentation cells, the outlet port of the first cell being in fluid communication with the inlet port of the second cell.

29. A microfabricated device as claimed in claim 28, further comprising a third fragmentation cell, the outlet port of the second cell being in fluid communication with the inlet port of the third cell.

30. A microfabricated device as claimed in claim 28 or claim 29 comprising a plurality of serially connected fragmentation cells.

31. A microfabricated device as claimed in any one of claims 28 to 30, wherein the size of the outlet port decreases the further down stream the fragmentation cell.

32. A microfabricated device as claimed in claim 31, wherein the size of the outlet port gradually decreases from the first fragmentation cell to the last fragmentation cell downstream.

33. A microfabricated device as claimed in any one of the preceding claims for fragmenting nucleic acids present in a biological fluid, a dairy product, an environmental fluid or drinking water.

34. A microfabricated reaction chamber system for carrying out a nucleic acid sequence amplification and detection process on a nucleic acid sample, the system comprising a microfabricated device as defined in any one of the preceding claims.

35. An apparatus for the analysis of biological and/or environmental samples, the apparatus comprising a device as

defined in any one of claims 1 to 33 or a system as defined in claim 34.

36. An assay kit for the analysis of biological and/or environmental samples, the kit comprising a device as defined in any one of claims 1 to 33 or a system as defined in claim 34 and means for contacting the sample with the device.

37. An apparatus as claimed in claim 35 or an assay kit as claimed in claim 36 which is disposable.

38. A process for fragmenting nucleic acids present in a fluid sample, the process comprising:

(a) providing a device as defined in any one of claims 1 to 33 or a microfabricated reaction chamber system as defined in claim 34 or an apparatus as defined in claim 35 or an assay kit as defined in claim 36;

(b) providing a fluid sample comprising nucleic acids;

(c) pumping the fluid sample into the inlet port of said device, through the fragmentation cell and out of the outlet port; and

(d) collecting the thus fragmented sample at the outlet port.

39. A process as claimed in claim 38 which further involves a nucleic acid sequence amplification and detection process on the fragmented nucleic acid sample.